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(54) Title: A METHOD FOR DETERMINING A MIMOTOPE SEQUENCE

(57) Abstract

The invention relates to a method for determining a mimotope sequence for a receptor comprising the steps of: a) providing a library of test sequences; b) determining the activity of each test sequence of the library towards the receptor; c) identifying a test sequence comprising at a certain position a building block which, according to the results of step b) is favored at said position; d) providing a next library of test sequences, based on said test sequence identified in step c), by replacing a building block at selected positions of the identified test sequence with selected building blocks; e) determining the activity of each test sequence of the library provided in step d) towards the receptor; f) identifying a test sequence comprising at a certain position a building block which, according to the results of step e), is favored at said position; g) repeating steps d) – f) for the library of test sequences provided in step d), for a number of cycles sufficient for finding in step f) a mimotope sequence that gives sufficient activity towards the receptor.

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Title: A method for determining a mimotope sequence

The invention relates to a method for determining a mimotope sequence.

Nowadays, random diversity libraries are widely used to identify lead molecules for diagnostics, pharmaca and vaccins. When the lead molecule is a peptide, the methods used for identifying the lead molecules are often referred to as pepscan methods.

Pepscan methods have been known since the early eighties. The basic theory behind these methods is described in EP-A-0 138 855 and EP-A-0 190 205.

Two pivotal aspects of screening of random diversity libraries are their size, i.e. how many different compounds are required to identify a lead molecule, and the method of optimization of the structure or sequence of the lead molecule. Most methods described in the literature seem to be based on the idea that the larger the random diversity library, the higher the chance of finding a good lead molecule.

The aspect with respect to which the known methods are most divergent, is the method of optimization of the lead molecule. Once the random diversity library has been synthesized, it can be tested for its desired activity. Of course it is possible to simply choose as a lead molecule the member of the random diversity library that scores the highest value for said desired activity. However, most methods comprise steps for optimizing the structure of said member that shows the highest activity, in order to arrive at a lead molecule that shows an even higher activity.

Minipepscan libraries, composed of only a few thousand peptides, have been used to identify lead molecules which were quickly optimized to molecules having an activity similar to that of molecules arrived at from starting from libraries composed of millions of compounds. This has been disclosed in Slootstra et al., Molecular Diversity, 1 (1995b) 87-96, and in Slootstra et al., J. Mol. Recogn., 10, 217-224

(1997). In these articles, it has been shown that small libraries in combination with pepscan-based optimization methods are a valuable tool in identifying and optimizing lead molecules.

The present invention aims to provide an improved method for identifying and optimizing a lead molecule.

It has been found that the objective improvement is achieved by starting from a library of known test molecules, evaluating the activity of said library, and by optimizing the structure of a few of test molecules showing the highest activity, by methodically replacing each building block of the structure of the test molecules with all other possible building blocks.

Thus, the invention relates to a method for determining a mimotope sequence for a receptor comprising the steps of:

a) providing a library of test sequences;
b) determining the activity of each test sequence of the library towards the receptor;
c) identifying a test sequence comprising at a certain position a building block which, according to the results of step b), is favored at said position;
d) providing a next library of test sequences, based on said test sequence identified in step c), by replacing a building block at selected positions of the identified test sequence with selected building blocks;
e) determining the activity of each test sequence of the library provided in step d) towards the receptor;
f) identifying a test sequence comprising at a certain position a building block which, according to the results of step e), is favored at said position;
g) repeating steps d) - f) for the library of test sequences provided in step d), for a number of cycles sufficient for finding in step f) a mimotope sequence that gives sufficient activity towards the receptor.

It has been found that the present method leads, in a convenient manner, to a mimotope sequence having a very high binding strength to the desired receptor. In some cases, where the exact epitope (i.e. the partial sequence of an antigen, that provides binding to an antibody) is known, the present method has been found to lead to the exact structure of the epitope in only a few steps.

According to the invention, a mimotope sequence for a receptor is determined. In this context, a mimotope sequence is defined as a molecule that shows a certain, minimal, desired activity in the presence of a given receptor. An example hereof is the determination of the epitope sequence of an antigen for a certain antibody. However, in some cases it may be sufficient if the exact epitope is mimicked and that a slightly less binding strength of the found molecule to the receptor suffices.

The mimotope sequence will be a molecule that is composed of a number of building blocks, wherein the number and order of building blocks controls the properties of the molecule. Examples of types of mimotope sequences that may be determined according to the invention include peptides, possibly having steroid or saccharide-like structures connected thereto, saccharides, DNA (oligonucleotides), and PNA (peptide-like nucleic acids). Thus, the building blocks will be chosen from the groups of amino acids (both natural and non-natural amino acids), monosaccharides, and nucleotides.

The receptor for which a mimotope sequences is determined according to the invention may be any compound, composition, microorganism, or tissue sample towards which one of the types of mimotope sequences may show some activity that can be measured, such as a binding activity. Suitable examples of receptors include antibodies (both monoclonal and polyclonal), proteins, such as enzymes, cells, hormone receptors, and micro-organisms.

The first step in a method according to the invention is the provision of a library of test sequences. Of course, these test sequences are composed of the same type or class of building blocks as the objective mimotope sequence. Preferably, the test sequences are known in that, although they may be randomly chosen, their composition and structure is known. This may be accomplished by generating, for instance by hand or by computer, a number of sequences and synthesizing the sequences thus obtained. It is also possible to derive the library of test sequences from a compound which is known to have a favorable activity towards the receptor. In principle, any known manner of selecting test sequences to make up the library is suitable.

The length of the test sequences is dependent on the nature of the building blocks constituting the sequences, and on the nature of the receptor and the desired activity towards said receptor. For most purposes, a length of between 3 and 20 building blocks will be suitable.

The number of test sequences constituting the library will be large enough to provide sufficient data to come to a good mimotope sequence in an acceptable number of steps/cycles. On the other hand, said number will be small enough to assure that the data obtained can be handled quite conveniently. Usually, the number of test sequences in the library will lie between 500 and 100,000, preferably between 1,000 and 10,000.

After the desired number of test sequences is generated, said test sequences may be synthesized. This may be done in any known manner, for instance as has been described by Slootstra et al. in Molecular Diversity, 1 (1995b), 87-96. Of course, it is also possible to make use of a library which is already available, for instance because it has been used in a previous run of the present method. In that case, the test sequences will not have to be synthesized.

For reasons of convenience, the test sequences are preferably synthesized using a minicard or a flat support medium as has been described in Dutch patent application 10.019703.

The next step of a method according to the invention is the determination of the activity of each test sequence of the library towards the receptor. The manner wherein this determination is carried out will depend on the specific interaction between mimotope sequence and receptor that is aimed at, and on the nature of the receptor and the building blocks of the mimotope sequence. For instance, when the desired activity is a binding of the mimotope sequence to the receptor, and the mimotope sequence is a peptide and the receptor is a monoclonal antibody, the determination may suitable be performed in an ELISA test, either in solution or on a solid support. Other suitable methods of determining the activity include BIACORE and AFM (Atomic Force Microscope). The skilled person will be able to choose a suitable manner of determination of the activity, given a certain receptor and nature of the mimotope sequence.

From the results of the determination of the activities of the test sequences of the library, at least one test sequence will be chosen to form a basis for the remaining steps of the present method. Said at least one test sequence is, in accordance with the invention, chosen by identifying at a certain position a building block which, as appears from the results of the determination of the activity, is favored at said position. In this regard, by the phrase "at a certain position a building block which is favored at said position" is meant that test sequences having at said position said building block show a high activity towards the receptor, relative to other activities found.

It is possible that the at least one test sequence which is identified was tested for its activity towards the receptor and showed itself a high activity, relative to other activities found.

It is also possible that the at least one test sequence which is identified, was in itself not tested. This can be explained as follows. For instance, it may have been found that a test sequence having at position 2 building block A shows a very high activity towards the receptor. It may further have been found that a test sequence having at position 4 building block B also shows a very high activity towards the receptor. The at least one test sequence to be identified may then be a sequence comprising building block A at position 2 and building block B at position 4. However, it may very well be that this sequence itself had not yet been evaluated for its activity towards the receptor.

Generally, the number of test sequences identified for basing the remaining steps of a method according to the invention on will be between 1 and 150. A higher number of test sequences chosen will lead to better results, but will be relatively more cumbersome to handle. Preferably, said number will be between 1 and 25. The skilled person will be able to decide on the number of test sequences chosen, dependent on the desired quality of the result of the method and the facilities available for carrying out the method.

On the basis of the identified test sequences, a next library of test sequences is provided in a so-called replacement analysis. This is done by varying the building blocks at positions of the identified test sequences, i.e. by replacing a building block at selected positions of the identified test sequence with selected building blocks.

For instance, in its simplest form the replacement analysis is carried out as follows. In case the test sequences are dodecapeptides of natural amino acids, first, the building block at position one of one of the chosen dodecapeptides may changed into each of the twenty naturally occurring amino acids, leading to 20 new test sequences. This is repeated for each position of the dodecapeptide, leading to 20 * 12 = 240 test sequences. Among these 240 sequences, the original dodecapeptide will be present 12 times. This

procedure is repeated for each chosen dodecapeptide. Thus, a next library is obtained which comprises from 240 to 3600 new test sequences.

More complex forms of this replacement analysis may be performed, for instance by allowing certain building blocks to be replaced by groups of building blocks (e.g. the replacement of one amino acid by two or more amino acids). Usually, such a replacement by multiple building blocks will be used sparingly. Preferably, in at least one replacement analysis of the different cycles of the present method, at least one building block is replaced by a group of building blocks. Care should be taken that in each replacement analysis not too many multiple building blocks are introduced, for then problems concerning the length of the sequence may arise. For the same reason, the multiple group of building blocks should preferably not be too long. The skilled person will, based on his experience in the field, be able to judge which size and number of multiple building blocks may be introduced.

It is also possible to replace a building block by a void in the replacement analysis. That way, the sequence will become one building block shorter. It will be clear that not too many building blocks should be replaced by a void at the same time, as this could lead to an undesirably short (or no) test sequence. The skilled person will be able to judge when and at which position it is helpful to introduce a void into a test sequence.

In addition, it is possible to allow the introduction of selected building blocks in the replacement analysis only, or to only replace building blocks at selected positions in the test sequences. Sometimes it is preferred to maintain certain building blocks at certain positions, e.g. in order to maintain a desired three dimensional structure of the test sequence. An advantage of this manner of performing the replacement analysis is that the total number of test sequences to be provided is limited. Generally, however, if

the building blocks at all positions of the test sequence are replaced by all possible building blocks, the test sequences having the desired three dimensional structure will also be provided. Again, it is within the skill of the artisan to judge in which cases it may be helpful to carry out a replacement at selected positions only. He will also be able to judge to which positions this may apply, and which building blocks may be used for the replacement and which building blocks may best not be used in the replacement (at certain positions).

The test sequences of the next library, which provided on the basis of the replacement analysis of the at least one identified test sequence, are tested for their activity towards the receptor. In case a test sequence is present among the test sequences of this library that shows sufficient activity towards the receptor, the method is completed and the desired mimotope sequence is obtained. It will be clear that it will depend on the type of activity, the receptor, the mimotope sequence, and the objective application of the mimotope sequence, whether a certain activity is regarded sufficient. Given the circumstances, the skilled person will be able to choose a threshold for a desired activity.

In case none of the test sequences shows sufficient activity towards the receptor, another cycle is carried out. In accordance with the invention, preferably at least two cycles, and thus two replacement analyses, are carried out. Thus, a next library of test sequences is provided, based on the 10 to 15 test sequences of the previous library that showed the highest activity, as set forth above. The test sequences in this next library will be evaluated for their activity, and so on.

The number of cycles that has to be carried out will depend on the desired degree of activity of the mimotope sequence to be found for the receptor. Starting from about 4500 randomly chosen dodecapeptides, it has been found

possible to arrive at the exact epitope sequence for a monoclonal antibody in less than three cycles.

In a preferred embodiment of the invention, the amount of receptor used for determining the activity of the test sequences towards said receptor, is lowered in each cycle. It has been found that building blocks that seem essential in the first cycle may become non-essential in further cycles. In other words, in said first cycle local optima may be found, which may be overcome in a subsequent cycle or in subsequent cycles. The lowering of the amount of the receptor in the determining of the activity of the test sequences has been found to assist in overcoming such local optima. Preferably, the amount of receptor used in the determination of the activities of test sequences in a library is reduced by a factor of from 50 to 1000, more preferably from 10 to 100.

The invention will now be further elucidated by the following, non-restrictive examples.

EXAMPLES

The described examples illustrate six variations of the present optimization method that has been successfully applied to lead peptides, identified with antibodies (monoclonal and polyclonal) raised against peptides, proteins, viruses, bacteria, sugars and steroids, from various types of peptide libraries. It can be envisaged that additional variations are possible. The concept that rules these variations is the repetition of replacement analyses on 1 or more lead peptides. Local minima are overcome and epitopes and mimotopes are identified.

Materials and Methods

Synthesis and screening minicards libraries (used in examples 1, 2, 5, 6)

Using the 20 natural L-amino acids 4550 random dodecapeptides (12-mers) were generated with a random generator programmed in Quick basic which runs on a 486 DX2 (66 MHz) computer system. In this library the frequency of each residue is approximately 5%. This set of sequences was used to design additional libraries. One in which all sequences are in the D-amino acid form, one in which the 2nd and 11th position are held by a cysteine and one in which the 3rd and 10th position are held by a cysteine. The aim of the latter two is to introduce a disulphide bridge into the peptide which should present the peptides as loops. Very important here is that the sequence of the amino acids within and outside the cysteines is identical to that of the first set of 4550 dodecapeptides. In this way the activity of these sets of dodecapeptides can be compared. **Example-1** describes results obtained with the synthetic peptide library one in which the 3rd and 10th position are held by a cysteine. **Example-2** describes results obtained with the synthetic peptide library composed of 4550 random dodecapeptides (12-mers), i.e. no cysteine or any other motif was used.

The libraries were synthesized and screened using credit-card format mini-PEPSCAN cards (455 peptides/card) as described previously (Slootstra et al., 1995b). In **example-1** the binding of monoclonal antibody 26/9 to each peptide was tested in a PEPSCAN-based enzyme-linked immuno assay (ELISA). Monoclonal antibody 26/9 has been described previously (Wilson et al., 1984; Rini et al., 1992; Churchill et al., 1994). Monoclonal antibody 26/9 was raised against the peptide HA175-110 of the hemagglutinin protein of influenza virus (X47:HA1) (Wilson et al., 1984).

In **example-1** the 455-well creditcard-format polyethylene cards, containing the covalently linked

peptides, were incubated with antibody 26/9 (100 μg/ml). After washing the peptides were incubated with rabbit-anti-mouse peroxidase (rampo, dilution 1/1000) (Dakopatts) (1 hr, 25oC), and subsequently, after washing the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 μl/ml 3% H2O2 were added. After 1 hr the color development was measured. The color development of the ELISA was quantified with a CCD-camera and an image processing system. The setup consists of a CCD-camera and a 55 mm lens (Sony CCD Video Camara XC-77RR, Nikon micro-nikkor 55 mm f/2.8 lens), a camara adaptor (Sony Camara adaptor DC-77RR) and the Image Processing Software package TIM, version 3.36 (Difa Measuring Systems, The Netherlands). TIM runs on a 486 DX2 (50 MHz) computer system.

The dodecapeptide library, composed of 4550 random dodecapeptides, is screened with relatively high concentration of antibody (100 μg/ml, more usually 10 μg/ml, see **examples 2-6**). This concentration is approximately 2 orders of magnitude above the concentration required to obtain maximal binding activity in ELISA with the native epitope peptide (not shown). The concentration of 100 μg/ml was used to obtain many binding peptides from the small set of 4550 random dodecapeptides. In previous studies it was shown that such a strategy, without obtaining an unfavorable signal to noise ratio, can result in hundreds of binding peptides that resemble small linear or non-linear parts of the native epitope (Slootstra et al., 1995b; Slootstra et al., 1997).

Synthesis and screening of OTHER pepscan libraries (used in example 4)

In addition to random minipepscan libraries mimotopes can also be identified from standard pepscan libraries. These libraries contain all overlapping 12-mers (or shorter/longer) covering the linear sequence of a known protein (Slootstra et al., 1995a).

Synthesis and screening of non-pepscan libraries (used in examples 3, 6)

Lead peptides can also be derived from other type of libraries such as for example phage-display libraries.

Sequence analysis of lead molecules (used in example-5)

All 4550 dodecapeptides were ranked according to their binding activity. According to their ranking consensus sequences and motifs are identified. Initial methods of lead optimization that only use the sequence of the top 50 molecules have been described in Slootstra et al.(1995b; 1997). The present method uses all 4550 sequences. Firstly, the frequency and/or distribution of single amino acids and the dipeptide motifs OO, dipeptide motifs OXO and dipeptide motifs OXXO is determined using Microsoft Excel 4.0 (O, one of the 20 natural L-amino acids; X, any residue). Secondly, properties of the amino acids (hydrophobicity, charge etc.) are included in the analysis. Thirdly, all this data is used to optimize the activity of the top 10-50 lead molecules. This is done by substitution of building blocks that inhibit activity and include building blocks that improve binding activity.

The present sequence analysis method improves the activity of lead molecules through motif analysis of all sequences part of the library.

Replacement analyses (used in examples 1, 2, 3, 4, 5, 6)

In a replacement analysis the binding activity of complete series of substitutions analogs of lead peptides, in which each position is replaced by each of the other 19 natural L-amino acids, is investigated in detail. In addition, it is possible to use the 20 D-amino acids as well as other available non-natural amino acids. Substitutions that result in improved binding are combined into new sequences of building blocks. These new sequences are again tested in a 2nd replacement analysis. If necessary more

rounds of replacement analyses are performed. After each round of a replacement analyses the antibody (or other soluble receptor) concentration can be lowered to obtain maximal binding activity (e.g. from 100 µg/ml to 0.01 µg/ml after 2 rounds of replacement-analyses, as shown in **example-1**).

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Notes for the examples:

- the 10 μg/ml, 1 μg/ml etc. denote the antibody concentration.

- The line "improved position-01" etc. means that this change in amino acid (is underlined) improves binding activity in pepscan elisa at given antibody concentration. Positions that could not be improved are not mentioned (e.g. position-08 in first replacement-net of example-1, i.e. the original I at position 8 gives the highest activity).

Example-1:

title: Identification of an epitope through a lead peptide selected from 4550 random dodecapeptides (minipepscan library with motif XXCXXXXXXCXX, X , randomly selected amino acid, cf. Materials and Method))

tools: lead peptide GCGAAMNIRCYA

methodology: two rounds of replacement analyses

Results:

In example-1, the lead peptide CGCAAMNIRCYA was derived (with antibody 26/9) from the random library in which the 3rd and 10th position are held by a cysteine (see above in Materials and methods). All possible single substitution analogs of the random peptide CGCAAMNIRCYA tested in pepscan for binding antibody 26/9 at 100 μg/ml. Below these results are described in detail.

The replacement analysis of the lead peptide CGCAAMNIRCYA resulted in the identification of building blocks that cannot be replaced by any other building blocks (e.g. Y and A), and to the identification of residues that can be replaced by one or two other building blocks (e.g. N), and to residues that can be replaced by many other building

blocks (e.g. M). Some replacements improve binding activity considerably (e.g. N into D).

All these replacements were used to design the improved peptide EMDEEEDIMNYA. Note that this peptide binds antibody 26/9 at much lower concentration, i.e. has improved binding activity (100 μg/ml for CGCAAMNIRCYA and 1.0 μg/ml for EMDEEEDIMNYA).

The peptide EMDEEEDIMNYA was run through a second replacement analysis. Again some replacements improve binding activity considerably. These replacements were used to design the improved peptide EMDEEEDVPDYA. Essential is that the first part of EMDEEEDIMNYA, EMDEEE, does not contain critical residues whereas the latter part, DIMNYA, does. Combination of the improved residues in this latter part results in the sequence DVPDYA. The sequence DVPDYA is identical to the linear epitope of antibody 26/9. Thus, the lead peptide CGCAAMNIRCYA derived from a few thousand random dodecapeptides was turned into native epitope sequence through two replacement analyses.

REPLACEMENT ANALYSIS-I

original lead: CGCAAMNIRCYA
activity at ≥ 100.0 μg/ml

improved position-01: EGCAAMNIRCYA
activity at ≥ 10.0 μg/ml

improved position-02: CMCAAMNIRCYA
activity at ≥ 10.0 μg/ml

improved position-03: CGDAAMNIRCYA
activity at ≥ 10.0 μg/ml

improved position-04: CGCEAMNIRCYA
activity at ≥ 10.0 μg/ml

improved position-05: CGCAEMNIRCYA
activity at ≥ 10.0 μg/ml

improved position-06: CGCAAENIRCYA
activity at ≥ 10.0 μg/ml

improved position-07: CGCAAMDIRCYA

activity at ≥ 10.0 μg/ml
improved position-09: CGCAAMNIMCYA
activity at ≥ 10.0 μg/ml
improved position-10: CGCAAMNIRNYA
activity at ≥ 10.0 μg/ml
combination improvements: EMDEEEDIMNYA
activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-II
combinations rep-an. I: EMDEEEDIMNYA
activity at ≥ 1.0 μg/ml
improved position-08: EMDEEEDVMNYA
activity at ≥ 0.1 μg/ml
improved position-09: EMDEEEDIPNYA
activity at ≥ 0.1 μg/ml
improved position-10: EMDEEEDIMDYA
activity at ≥ 0.1 μg/ml
combination improvements: EMDEEEDVPDYA
activity at ≥ 0.01 μg/ml

Notes:

The sequence DVPDYA is the original epitope. The peptide EMDEEEDVPDYA has a 10-fold improved binding affinity (in solution) over the native epitope peptide YPYDVPDYASLRS.

Example-2:

title: Identification of a mimotope through a lead peptide selected from 4550 random dodecapeptides (random minipepscan library)

tools: lead peptide ANWPSAIGAFGL

methodology: three rounds of replacement analyses

Results:

In example-2, lead peptides were identified through a random minipepscan library. The difference of example-2 with example-1 is that the antibody used in example-1 binds a linear epitope whereas the antibody used in example-2 binds a non-linear epitope. The following multiple replacement analyses were done as discussed in example-1. The identified mimotope does not resemble any region of the linear sequence of the native protein.

REPLACEMENT ANALYSIS-I

original lead:	ANWPSAIGAFGL
	activity at ≥ 10.0 μg/ml
improved position-01:	HNWPSAIGAFGL
	activity at ≥ 5.0 μg/ml
improved position-02:	AWWPSAIGAFGL
	activity at ≥ 5.0 μg/ml
improved position-03:	ANAPSAIGAFGL
	activity at ≥ 5.0 μg/ml
improved position-04:	ANWSSAIGAFGL
	activity at ≥ 5.0 μg/ml
improved position-11:	ANWSSAIGAFKL
	activity at ≥ 5.0 μg/ml
combination improvements:	HWASSAIGAFKL
	activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-II

combinations rep-an. I:	HWASSAIGAFKL
	activity at ≥ 1.0 μg/ml
improved position-01:	KWASSAIGAFKL
	activity at ≥ 0.5 μg/ml
improved position-02:	HYASSAIGAFKL
	activity at ≥ 0.5 μg/ml
improved position-03:	HWGSSAIGAFKL
	activity at ≥ 0.5 μg/ml
improved position-07:	HWASSAMGAFKL

activity at ≥ 0.5 μg/ml

combination improvements: KYGSSAMGAFKL

activity at ≥ 0.1 μg/ml

REPLACEMENT ANALYSIS-III

combinations rep-an. II: KYGSSAMGAFKL

activity at ≥ 0.1 μg/ml

improved position-03: KYFSSAMGAFKL

activity at ≥ 0.05 μg/ml

improved position-06: KYGSSGMGAFKL

activity at ≥ 0.05 μg/ml

combination improvements: KYFSSGMGAFKL

activity at ≥ 0.01 μg/ml

Example-3:

title: Identification of a mimotope through a lead derived from phage-display library composed of >1000.000 random hexapeptides.

tools: lead peptide SDTRKG*

methodology: lengthening to the left and to the right of SDTRKG with cysteines/glycines, followed by three rounds of replacement-analyses

Results:

In example-3, lead peptides were identified through phage-display. Adjacent cysteines/glycines were added to improve binding activity. After obtained activity multiple replacement analyses were done as discussed in example-1.

LENGTHENING BY CYSTEINES/GLYCINES:

phage-display lead:	SDTRKG
	NO activity at 10.0 μg/ml
lengthened lead:	CSDTRKGC
	activity at ≥ 10.0 μg/ml
lengthened lead:	CSDTRKGCG
	activity at ≥ 10.0 μg/ml

REPLACEMENT ANALYSIS-I

lengthened lead:	CSDTRKGCG
	activity at ≥ 10.0 μg/ml
improved position-02:	C<u>T</u>DTRKGCG
	activity at ≥ 5.0 μg/ml
improved position-03:	CS<u>E</u>TRKGCG
	activity at ≥ 5.0 μg/ml
improved position-05:	CSDT<u>H</u>KGCG
	activity at ≥ 5.0 μg/ml
improved position-06:	CSDTR<u>Y</u>GCG
	activity at ≥ 5.0 μg/ml
combination improv.:	C<u>TE</u>T<u>HY</u>GCG
	activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-II

comb. rep-an. I:	CTETHYGCG
	activity at ≥ 1.0 μg/ml
improved position-02:	C<u>Y</u>ETHYGCG
	activity at ≥ 0.5 μg/ml
improved position-05:	CTET<u>K</u>YGCG
	activity at ≥ 0.5 μg/ml
improved position-06:	CTETH<u>F</u>GCG
	activity at ≥ 0.5 μg/ml
combination improv.:	C<u>Y</u>ET<u>KF</u>GCG
	activity at ≥ 0.1 μg/ml

REPLACEMENT ANALYSIS-III

comb. rep-an. II:	CYETKFGCG
	activity at $\geq$ 0.1 μg/ml
improved position-01:	DYETKFGCG
	activity at $\geq$ 0.05 μg/ml
improved position-08:	CYETKFGNG
	activity at $\geq$ 0.05 μg/ml
combination improv.:	DYETKFGNG
	activity at $\geq$ 0.01 μg/ml

*, this lead peptide is, as a synthetic peptide, not active (not in elisa (pepscan or standard nor in solution). This is not unique. Often phage-peptides are only active as part of the phage-coat protein. In other formats they lose their activity.

Example-4:

title: Identification of a mimotope through a lead derived from a standard pepscan analysis.

tools: lead peptide RVMIKLILVNFR* and complete sequence of native protein (part which was used is KIYRVMIKLILVNFRMQP).

methodology: Two rounds of replacement-analyses, followed by lengthening to the left and right, again followed by two more rounds of replacement-analyses, finally followed by lenthening to an 18-mer mimotope.

Results:

In example-4, a lead peptide was identified through standard pepscan analysis, i.e. the antibody was tested on all overlapping 12-mers covering the linear sequence of the protein. The following two rounds of replacement analyses were done as discussed in example-1. In addition, the

mimotope was lengthened to the left and right (3 amino acids, this can be further lengthened) through additional replacement analyses.

REPLACEMENT ANALYSIS-I

original lead:	RVMIKLILVNFR
	activity at ≥ 10.0 μg/ml
improved position-01:	AVMIKLILVNFR
	activity at ≥ 5.0 μg/ml
improved position-02:	AIMIKLILVNFR
	activity at ≥ 5.0 μg/ml
improved position-03:	AVPIKLILVNFR
	activity at ≥ 5.0 μg/ml
improved position-08:	AVMIKLIRVNFR
	activity at ≥ 5.0 μg/ml
improved position-11:	AVMIKLILVNYR
	activity at ≥ 5.0 μg/ml
combination improv.:	AIPIKLIRVNYR
	activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-II

comb. rep-an. I:	AIPIKLIRVNYR
	activity at ≥ 1.0 μg/ml
improved position-01:	YIPIKLIRVNYR
	activity at ≥ 0.5 μg/ml
improved position-02:	APPIKLIRVNYR
	activity at ≥ 0.5 μg/ml
combination improv.:	YPPIKLIRVNYR
	activity at ≥ 0.1 μg/ml

LENTHENING WITH NATIVE SEQUENCE:

comb. rep-an. II:	YPPIKLIRVNYR
	activity at ≥ 0.1 μg/ml
lengthened left:	KIYYPPIKLIRV
	activity at ≥ 1.0 μg/ml
lengthened right:	IKLIRVNYRMQP

activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-III of left lengthened peptide:

lengthened left:	KIYYPPIKLIRV
	activity at ≥ 1.0 μg/ml
improved posit-01:	RIYYPPIKLIRV
	activity at ≥ 0.5 μg/ml
improved posit-02:	KPYYPPIKLIRV
	activity at ≥ 0.5 μg/ml
improved posit-03:	KIWYPPIKLIRV
	activity at ≥ 0.5 μg/ml
improved posit-08:	KIYYPPISLIRV
	activity at ≥ 0.5 μg/ml
combination impr.:	RPWYPPISLIRV
	activity at ≥ 0.1 μg/ml

REPLACEMENT ANALYSIS-IV of right lengthened peptide:

lengthened right:	IKLIRVNYRMQP
	activity at ≥ 1.0 μg/ml
improved posit-10:	IKLIRVNYRCQP
	activity at ≥ 0.5 μg/ml
improved posit-11:	IKLIRVNYRMEP
	activity at ≥ 0.5 μg/ml
improved posit-12:	IKLIRVNYRMQN
	activity at ≥ 0.5 μg/ml
combination impr.:	IKLIRVNYRCEN
	activity at ≥ 0.1 μg/ml
combination total:	RPWYPPISLIRVNYRCEN
	activity at ≥ 0.01 μg/ml

*, lead peptide was identified from a library composed of all overlapping 12-mers covering the linear sequence of a protein. This makes it possible to lengthen the peptide to the left and right with adjacent amino acids, in this case KIY on the left and MQP on the right. Additional replacement-

analyses of 12-mers, shifted three to the left or three to the right of the sequence finally results in an 18-mer mimotope.

Example-5:

title: Identification of a mimotope through a set of similar leads selected from 4550 random dodecapeptides (random minipepscan library)

tools: set of 6 lead peptides QNNMKLFRGCVP, RGIKWNEMTDQW, KLQQNPTFYPPV, TNNCKEFAGIVP, RGILTNIMKDQW, IVQNNPKFFRGA (potentially up to all 4550 peptides, see material and method, in this case also 'negative' amino acids are removed from the leads)

methodology: : determination of consensus sequence, followed by two rounds of replacement analyses of the consensus sequence

Results:

In example-5, a set of similar lead peptides were used to identify a consensus sequence. The consensus sequences were identified as discussed in materials and method. This sequence was used in the replacement analyses.

ALIGNMENT OF LEAD PEPTIDES:

```
      QNNMKLFRGCVP
RGIKWNEMTDQW
  KLQQNPTFYPPV
    TNNCKEFAGIVP
RGILTNIMKDQW
  IVQNNPKFFRGA
```

consensus: ILQNNMKDFRG

REPLACEMENT ANALYSIS-I

consensus lead:	ILQNNMKDFRG activity at ≥ 1.0 μg/ml
improved position-03:	ILTNNMKDFRG activity at ≥ 0.5 μg/ml
improved position-07:	ILQNNMPDFRG activity at ≥ 0.5 μg/ml
improved position-10:	ILQNNMKDWRG activity at ≥ 0.5 μg/ml
combination improv.:	ILTNNMPDWRG activity at ≥ 0.1 μg/ml

REPLACEMENT ANALYSIS-II

comb. rep-an. I:	ILTNNMPDWRG activity at ≥ 0.1 μg/ml
improved position-07:	ILTNNMGDWRG activity at ≥ 0.05 μg/ml
improved position-11:	ILTNNMPDWYG activity at ≥ 0.05 μg/ml
combination improv.:	ILTNNMGDWYG activity at ≥ 0.01 μg/ml

Example-6:

title: Identification of a mimotope through a set of different leads selected from phage-display library composed of >1,000,000 random hexapeptides.

tools: set of 3 lead peptides (ANWPSA, KLITRW, NVCSWS)

methodology: Two rounds of replacement analyses (for each lead peptide), followed by determination of overall consensus sequence.

Results:

In example-6, different lead peptides were used to identify a consensus sequence. Each lead peptide was used in multiple replacement analyses (two rounds). The resulting three mimotopes were aligned which resulted in a lengthened mimotope with improved activity.

REPLACEMENT ANALYSIS-IA

original lead	: ANWPSA	: activity at ≥10.0	µg/ml
improved position-01:	HNWPSA	: activity at ≥ 5.0	µg/ml
improved position-02:	AWWPSA	: activity at ≥ 5.0	µg/ml
improved position-03:	ANAPSA	: activity at ≥ 5.0	µg/ml
improved position-04:	ANWSSA	: activity at ≥ 5.0	µg/ml
combination improv. :	HWASSA	: activity at ≥ 1.0	µg/ml

REPLACEMENT ANALYSIS-IIA

comb. rep-an. IA	: HWASSA	: activity at ≥ 1.0	µg/ml
improved position-05:	HWASPA	: activity at ≥ 0.5	µg/ml
combination improv. :	HWASPA	: activity at ≥ 0.5	µg/ml

REPLACEMENT ANALYSIS-IB

original lead	: KLITRW	: activity at ≥10.0	µg/ml
improved position-01:	SLITRW	: activity at ≥ 5.0	µg/ml
improved position-02:	KSITRW	: activity at ≥ 5.0	µg/ml
improved position-03:	KLATRW	: activity at ≥ 5.0	µg/ml
improved position-06:	KLITRY	: activity at ≥ 5.0	µg/ml
combination improv. :	SSATRY	: activity at ≥ 1.0	µg/ml

REPLACEMENT ANALYSIS-IIB

comb. rep-an. IB	: SSATRY	: activity at ≥ 1.0	µg/ml
improved position-02:	SPATRY	: activity at ≥ 0.5	µg/ml
combination improv. :	SPATRY	: activity at ≥ 0.5	µg/ml

REPLACEMENT ANALYSIS-IC

original lead	: NVCSWS	: activity at ≥10.0	µg/ml
improved position-02:	NICSWS	: activity at ≥ 5.0	µg/ml

improved position-04: NVCHWS : activity at ≥ 5.0 μg/ml
improved position-06: NVCSWA : activity at ≥ 5.0 μg/ml
combination improv. : NICHWA : activity at ≥ 1.0 μg/ml

REPLACEMENT ANALYSIS-IIC
comb. rep-an. IC : NICHWA : activity at ≥ 1.0 μg/ml
improved position-01: YICHWA : activity at ≥ 0.5 μg/ml
improved position-02: NVCHWA : activity at ≥ 0.5 μg/ml
combination improv. : YVCHWA : activity at ≥ 0.1 μg/ml

ALIGNMENT combination improv. IIA, IIB and IIC:

combination improvements 1: HWASPA
combination improvements 2: SPATRY
combination improvements 3: YVCHWA
consensus: YVCHWASSATRY
activity at 0.01 μg/ml

Claims

1. A method for determining a mimotope sequence for a receptor comprising the steps of:

a) providing a library of test sequences;

b) determining the activity of each test sequence of the library towards the receptor;

c) identifying a test sequence comprising at a certain position a building block which, according to the results of step b), is favored at said position;

d) providing a next library of test sequences, based on said test sequence identified in step c), by replacing a building block at selected positions of the identified test sequence with selected building blocks;

e) determining the activity of each test sequence of the library provided in step d) towards the receptor;

f) identifying a test sequence comprising at a certain position a building block which, according to the results of step e), is favored at said position;

g) repeating steps d) - f) for the library of test sequences provided in step d), for a number of cycles sufficient for finding in step f) a mimotope sequence that gives sufficient activity towards the receptor.

2. A method according to claim 1, wherein the chemical composition of the test sequences provided in step a) is known.

3. A method according to claim 1 or 2, wherein in step e) an amount of receptor is used for determining the activity, which amount is smaller than said amount used in step b), and wherein said amount in step g) is smaller than said amount in step e) of the cycle directly preceding said step g).

4. A method according to claim 3, wherein in step e) the amount of receptor used for determining the activity is smaller by a factor in the range of from 5 to 1000 smaller than said amount used in step b), and wherein said amount in

step g) is smaller by a factor in the range of from 5 to 1000 than said amount in step e) of the cycle directly preceding said step g).

5. A method according to claim 4, wherein in step e) the amount of receptor used for determining the activity is smaller by a factor in the range of from 10 to 100 smaller than said amount used in step b), and wherein said amount in step g) is smaller by a factor in the range of from 10 to 100 than said amount in step e) of the cycle directly preceding said step g).

6. A method according to any one of the preceding claims, comprising at least one step d) wherein at least one building block is replaced by a group of building blocks.

7. A method according to any one of the preceding claims, wherein the predetermined set of building blocks comprises amino acids, monosaccharides, and nucleotides.

8. A method according to any one of the preceding claims, wherein the test sequences comprise from 3 to 20 building blocks.

9. A method according to any one of the preceding claims, wherein the library of test sequences of step a) comprises from 500 to 10,000 test sequences.

10. A method according to any one of the preceding claims, wherein the receptor is chosen from the group consisting of monoclonal antibodies, proteins, such as enzymes, cells, hormone receptors, and micro-organisms.

11. A method according to any one of the preceding claims, wherein the activity is determined using an immuno assay, BIACORE or AFM.

12. A method according to any one of the preceding claims, wherein each test sequence of a library is physically separated from the other test sequences of said library.

13. A method according to claim 12, wherein each test sequence is located on a minicard or flat support medium.

14. A mimotope sequence obtainable in a method according to any one of the preceding claims.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/NL 99/00700

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C07K1/04 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 26775 A (HOUGHTEN PHARM INC) 24 November 1994 (1994-11-24) claims 18-26	1-14
X	US 5 814 470 A (BLONDELLE SYLVIE ET AL) 29 September 1998 (1998-09-29) claims 6-9 column 20, line 46 -column 23, line 17	1-14
X	US 5 763 193 A (OSTRESH JOHN M ET AL) 9 June 1998 (1998-06-09) claims column 30, line 42 -column 32, line 65	1-14
X	US 5 645 996 A (BLONDELLE SYLVIE ET AL) 8 July 1997 (1997-07-08) column 20, line 17 -column 23, line 42	1-14

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2000	07/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Routledge, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 582 997 A (BLONDELLE SYLVIE ET AL) 10 December 1996 (1996-12-10) column 17, line 39 -column 21, line 20	1-14
X	US 5 565 325 A (BLAKE JAMES) 15 October 1996 (1996-10-15) claims column 3, line 15 -column 5, line 32	1-14
X	US 5 556 762 A (PINILLA CLEMENCIA ET AL) 17 September 1996 (1996-09-17) claims 5-11 column 7, line 31 -column 8, line 37 column 33, line 64 -column 35, line 18	1-14
X	HALE G: "Synthetic peptide mimotope of the CAMPATH-1 (CD52) antigen, a small glycosylphosphatidylinositol-anchored glycoprotein" IMMUNOTECHNOLOGY, vol. 1, no. 3, 1 December 1995 (1995-12-01), page 175-187 XP004052720 page 179, right-hand column, paragraph 1 -page 181, right-hand column, paragraph 1 page 186, left-hand column, paragraph 2	1-14
X	DE KOSTER H S ET AL: "The use of dedicated peptide libraries permits the discovery of high affinity binding peptides" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 187, no. 1, 16 November 1995 (1995-11-16), page 179-188 XP004020980 page 183, left-hand column, paragraph 3 -page 185, left-hand column, paragraph 2; table 2	1,2, 6-10,12, 14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/NL 99/00700

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		WO 9209300 A	11-06-1992

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 18 July 2000 (18.07.00)	
International application No. PCT/NL99/00700	**Applicant's or agent's file reference** P21526PC00
International filing date (day/month/year) 15 November 1999 (15.11.99)	**Priority date** (day/month/year) 13 November 1998 (13.11.98)
Applicant SLOOTSTRA, Jelle, Wouter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 June 2000 (08.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PTO/PCT Rec'd 14 JUN 20[redacted]

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To:
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PAYS-BAS

MRF 13-5-2001

Sup rapp

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

- 5 JUNE 2000 47.1(c), first sentence)

Date of mailing (day/month/year)	
25 May 2000 (25.05.00) cl.2916 dd.	
Applicant's or agent's file reference P21526PC00	**IMPORTANT NOTICE**

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/NL99/00700	15 November 1999 (15.11.99)	13 November 1998 (13.11.98)

Applicant
STICHTING DIENST LANDBOUWKUNDIG ONDERZOEK et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,MA,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH, GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL, PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   25 May 2000 (25.05.00) under No. WO 00/29851

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des C lombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

TENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
KB P21526PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL99/00700	15/11/1999	13/11/1998
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant STICHTING DIENST LANDBOUWKUNDIG ONDERZOEK et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08/06/2000	13.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Routledge, B Telephone No. +31 70 340 4272

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL99/00700

I. Basis f the r p rt

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-26	as originally filed				
Claims, No.:					
1-12	as received on	12/01/2001	with letter of	12/01/2001	

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: **WO 94/26775 A**
D2: **US 5 814 470 A**
D3: **US 5 763 193 A**
D4: **US 5 645 996 A**
D5: **US 5 582 997 A**
D6: **US 5 565 325 A**
D7: **US 5 556 762 A**
D8: **Immunotechnology (1995) 1 175-187**
D9: **J.Immunol.Mtds (1995) 187 179-188**

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application satisfies the criteria of requirements of Article 33(2) PCT, with regard to novelty. None of the documents cited on the International Search Report discloses all the features of claim **1**, namely a method for determining a mimotope sequence to a receptor comprising

   (a) solid support with a random library of sequences, said sequences consisting of amino acids, monosaccharides or nucleotides as building blocks, determining activity and identifying sequences having a building block at a favoured position,

   (b) constructing a library from said sequences, analysing for activity and identifying sequences having a building block at a favoured position,

   (c) constructing a further library based upon the identifed sequences in (b) and repeating step (b) until a sequence (mimotope) having the desired activity to said receptor is identified

   wherein all the sequences are located on a minicard or flat support .

2. The present application does not meet the requirements of Article 33(3)PCT because the subject matter of claims **1-12** lacks an inventive step. The provision of a first library, analysis of a reaction (activity) and construction of a further library or libraries based upon the results obtained is well known in the state of the art and demonstrated in **D2 (claims)**, **D4** (col. **20-23**), **D5** (col. **17-21**), **D6 (claims)** , **D7 (claims)**, **D8** (pages **179-181**, **186**) and **D9** (pages **183-185**). The only two features which distinguish the present application from the state of the art are the use of a random library and the fact that the test squences are attached to a solid support (minicard). Both of these features are well known in the prior art as demonstrated by **D6** (claim **1**), **D8** (page **176**, right hand column, **2nd** paragraph) which also provides further references ("..29-31.") and **D9** (page **180**, **184**) with regard to the use of random libraries and **D5** (col. **17** lines **25-38**) and **D8** (section **2.4**). Neither of these features renders the subject matter of the claims inventive over the cited prior art.

2.1 Thus the inventive contribution lies in the mere repetition of the same steps until a sequence is obtained that meets the requirements of the problem i.e. produces optimised activity. Such repetition falls within the knowledge and ability of the skilled person and does not constitute an inventive step. The mere production of a mimotope sequence by a non-inventive method (independent claim **12**) similarly lacks an inventive step for the same reasoning as mimotope sequences per se are known. Dependent claims **2-11** do not appear to contain any additional features which, in combination with the features of any claim to which they refer meet the requirements of the PCT with respect to inventive step as the embodiments are either disclosed in the cited prior art or also fall within the knowledge and ability of the skilled person.

2.2 **D1** and **D3** refer to chemical compound synthesis from building blocks other than amino acids, monosaccharides or nucleotides and are therefore not relevant to the subject matter of the claims.

3. All claims meet the criteria of Article 33(4) PCT with regard to industrial applicability.

R Item VIII

Certain observations on the international application

1. The application does not meet the requirements of Article 6 PCT for the following reasons:-

   (a) The subject matter of the claims is vague and obscure due to the use of such phrases as "..at a certain position..", "..is favoured at..", "..sufficient for.." or "..sufficient activity.." which all lead to a lack of clarity.

   (b) The scope of the claims is unduly broad referring to the use of "building blocks" which may be amino acids, monosaccharides or nucleotides (claim **1**). However the description only provides adequate technical support for the use of amino acids, being wholly silent with regard to the remaining "building blocks" other than a simple restatement of the claim (page **3**).

   (c) The use of the relative term "..smaller.." in claim **3** leads to a lack of clarity.

   (d) Claim **4** is inconsistent with page **9** lines **13-16** and claim **8** is inconsistent with page **4** lines **24-26**.

   (e) Claim **11** lacks support in the body of the description.

   (f) Claim **12** is lacking in clarity and is unduly broad as the claim is not limited by any technical (sequences) or functional characteristics.

   (g) The sequence at line **19** page **14** is incorrect and inconsistent with the rest of Example **1**.

2. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents **D2**, **D4-D9** is not mentioned in the description, nor are these documents identified therein.

New Claims

1. A method for determining a mimotope sequence for a receptor comprising the steps of:

a) providing a solid support with a random library of test sequences composed of building blocks chosen from the group of amino acids, monosaccharides and nucleotides;

b) determining the activity of each test sequence of the library towards the receptor;

c) identifying a test sequence comprising at a certain position a building block which, according to the results of step b), is favored at said position;

d) providing a next library of test sequences, based on said test sequence identified in step c), by replacing a building block at selected positions of the identified test sequence with selected building blocks;

e) determining the activity of each test sequence of the library provided in step d) towards the receptor;

f) identifying a test sequence comprising at a certain position a building block which, according to the results of step e), is favored at said position;

g) repeating steps d) - f) for the library of test sequences provided in step d), for a number of cycles sufficient for finding in step f) a mimotope sequence that gives sufficient activity towards the receptor;

wherein each test sequence is located on a minicard or flat support medium.

2. A method according to claim 1, wherein the chemical composition of the test sequences provided in step a) is known.

3. A method according to claim 1 or 2, wherein in step e) an amount of receptor is used for determining the activity, which amount is smaller than said amount used in step b), and wherein said amount in step g) is smaller than said amount in step e) of the cycle directly preceding said step g).

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New page 2

4. A method according to claim 3, wherein in step e) the amount of receptor used for determining the activity is smaller by a factor in the range of from 5 to 1000 smaller than said amount used in step b), and wherein said amount in step g) is smaller by a factor in the range of from 5 to 1000 than said amount in step e) of the cycle directly preceding said step g).

5. A method according to claim 4, wherein in step e) the amount of receptor used for determining the activity is smaller by a factor in the range of from 10 to 100 smaller than said amount used in step b), and wherein said amount in step g) is smaller by a factor in the range of from 10 to 100 than said amount in step e) of the cycle directly preceding said step g).

6. A method according to any one of the preceding claims, comprising at least one step d) wherein at least one building block is replaced by a group of building blocks.

7. A method according to any one of the preceding claims, wherein the test sequences comprise from 3 to 20 building blocks.

8. A method according to any one of the preceding claims, wherein the library of test sequences of step a) comprises from 500 to 10,000 test sequences.

9. A method according to any one of the preceding claims, wherein the receptor is chosen from the group consisting of monoclonal antibodies, proteins, such as enzymes, cells, hormone receptors, and micro-organisms.

10. A method according to any one of the preceding claims, wherein the activity is determined using an immuno assay, BIACORE or AFM.

11. A method according to any one of the preceding claims, wherein each test sequence of a library is physically separated from the other test sequences of said library.

12. A mimotope sequence obtainable in a method according to any one of the preceding claims.

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IPEA/EP

M.H

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P21526PC00		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 99/ 00700	15/11/1999	13/11/1998

Applicant

STICHTING DIENST LANDBOUWKUNDIG ONDERZOEK et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure N .

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.